Exploring the Ecological Distribution and Antibiotic Resistance Profiles of Klebsiella Species in Diverse Environmental Niches

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Abstract: Antimicrobial resistance is a growing problem in bacterial pathogens, particularly in hospital-acquired nosocomial infections. Klebsiella is an important pathogen that causes various infections in hospitalized immunocompromised patients. Therefore, this study aims to fill this knowledge gap and provide insights into the correct detection and antibiotic resistance pattern of these organisms. We will investigate the spread of pneumonia bacteria, Klebsiella, isolate and identify it from different environmental sites, including human, soil, and water, also study the characteristics of antibiotic resistance and find similarities and differences between bacterial isolates. Finally, we detect the associated antibiotics resistance gene (CTX-M), using the genetic RT-PCR method. The study provides valuable information on the prevalence of antibiotic resistant Klebsiella spp. in different clinical and environmental sites, which can help in the development of effective strategies to control the spread of these bacteria. The study found that among all 86 isolated strains of Klebsiella spp. it was found that K. pneumoniae represents the major abundant strain in both clinical and environmental samples by percentages 41.5% and 51.5% respectively. It concerns also the highest ESBL production 71.7%, along with 58% CTX-M gene expression.

Keywords: Klebsiella species, Klebsiella pneumoniae, Drug resistance, CTX-M.

Introduction

Klebsiella species (Klebsiella spp.) refers to a group of bacteria belonging to the genus Klebsiella. This genus is the second most populous enteric genus found in the gastrointestinal tract of the human. Klebsiella bacteria are Gram-negative, non-motile, rod-shaped bacteria. While some Klebsiella species are harmless and considered a part of the normal human flora, certain strains can cause a wide range of infection. Klebsiella spp. are notable for their ability to develop resistance to multiple antibiotics, making them a significant concern in healthcare settings and communities worldwide [1].

Klebsiella spp. are amongst the most frequently common causes of a wide range of infections both community-acquired and hospital-acquired including, Urinary tract infections (UTIs), Pneumonia, Wound infections, Bloodstream infections, Surgical site infections and Respiratory tract infections [2].

It is important to note that the severity and clinical manifestations of these infections can vary depending on various factors including the individual's overall health, the specific Klebsiella strain
involved, and the site of infection. The emergence and spread of antibiotic resistant Klebsiella strains highlight the importance of appropriate antibiotic stewardship practices, infection control measures, and the development of new treatment strategies to combat these infections effectively [3].

The Centers for Disease Control (CDC), and Bergey's Manual of Determinative Bacteriology, recognized four Klebsiella species, as Klebsiella pneumoniae (K. pneumoniae), Klebsiella oxytoca (K. oxytoca), Klebsiella planticola (K. planticola) and Klebsiella terrigena (K. terrigena) [4, 5].

Many Klebsiella strains produce enzymes known as extended-spectrum beta-lactamases (ESBLs). These enzymes can inactivate a broad range of beta-lactam antibiotics, including penicillins and cephalosporins, rendering them ineffective in treating infections which is now considered a critical concern for therapies development against bacterial infection. Klebsiella spp. also produce AmpC beta-lactamase enzymes, which can confer resistance to certain cephalosporins and beta-lactam antibiotics[6]. AmpC-producing Klebsiella strains can pose challenges in treatment, especially when combined with other resistance mechanisms [7, 8].

Besides beta-lactam antibiotics, Klebsiella spp. can exhibit resistance to other classes of antibiotics, including fluoroquinolones, aminoglycosides, and sulfonamides. This multidrug resistance can severely limit treatment options [9].

Furthermore, CTX-M (Cefotaximase-Munich) beta-lactamases are a group of ESBLs that are commonly found in various Enterobacteriaceae, including Klebsiella species. These enzymes cause resistance to third generation cephalosporins, as cefotaxime and ceftriaxone, and are a significant concern in healthcare settings due to their ability to render these antibiotics ineffective. CTX-M-type ESBLs have been detected in various strains of Klebsiella species, including Klebsiella pneumoniae, Klebsiella oxytoca, and Klebsiella planticola. These enzymes are often responsible for mediating resistance to cephalosporins and can contribute to the challenge of treating infections caused by these bacteria [10].

The study investigates the prevalence of antibiotic resistant Klebsiella spp. in different environmental sites and to develop effective strategies to control the spread of these bacteria.

Methods
Study design and isolates bacteria

The bacterial collection consisted of 86 Klebsiella spp. isolated during the period from 1st December 2022 to 15th March 2023. Clinical isolates (n = 53) were obtained from patients (aged > 20 years) with positive samples processed by the microbiology diagnostic laboratory, comprising 36 urine samples and 17 sputum samples obtained from Al-Zahra Teaching Hospital, in Al-Kut city. Date of isolation and sample type detected, and samples were de-duplicated so that only one patient isolation was included.

Environmental isolates and Wastewater (n = 33) were obtained through a cross-sectional survey between December 2022 and March 2023. Wastewater samples were collected from 20 canals and 13 soil areas within the surrounding area of Al-Zahra Hospital.

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Identification of different Species and antibiotic sensitivity tests of Klebsiella spp. The identification of Klebsiella spp. collected samples incubated in LB broth for 16–24 h then it was conducted by culturing on McConkey agar, Eosin Methylene Blue (EMB) agar plates and biochemical testing using API 20E kit (BioMerieux, Inc.). The Kirby Bauer method was used to perform antibiotic sensitivity tests.

Detection of Bacterial Isolates Producing Extended spectrum beta-lactamase (ESBL) Enzymes

Disk approximation test used to assess the presence of ESBLs and AmpC β-lactamase enzymes in bacterial isolates. This technique involves the use of specific antibiotic disks that help determine the production of these enzymes. ESBL detection performed as mentioned by the CLSI method, which using ceftazidime (30 μg) and cefotaxime (30 μg) discs alone and in combination with clavulanic acid discs. controls for the study K. pneumoniae (ATCC-700603) disks were used. The confirmation of ESBL production was done by MicroScan MIC 37 panel using combination of ceftazidime/K clavulanate (Caz/CA) and cefotaxime/K clavulanate (Cft/CA).

Detection of Bacterial Isolates Producing metallo beta-lactamase (MBL) Enzymes

The Modified Hodge test was employed to detect the presence of Metallo-β-lactamase (MBL) enzymes in bacterial isolates. This test involves the evaluation of bacterial growth around a carbapenem-containing disk, which indicates the potential production of MBL enzymes.

Detected the presence of CTX-M gene by RT-PCR.

Three hundred milligrams of 24- to 72-h-old agar culture were prepared for extraction. The total genomic bacterial RNA of each isolated Klebsiella spp. was isolated using a Quick-RNA Miniprep Plus Kit (Zymo, Cat # R1058) the primers were designed by using Primer Premier 5.0. Purified total (1 μg) RNA was subjected to reverse transcription with reverse transcriptase kit High-capacity CDNA Reverse Transcription (Applied Biosystems, Cat # 4368813) according to the manufacturer's instructions. Relative amounts of each transcript were calculated using the ΔΔCt method using gapA (glyceraldehyde-3-phosphate dehydrogenase) as a control.

Table 1. Primers used in the study.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer</th>
</tr>
</thead>
</table>
| CTX-M       | F: 5’-ATGAAATTGCGTCAGTG-3’  
              | R: 5’-TTACTGCGATATCGTTGTC-3’ |
| gapA        | F: 5’-ATGTCGTGGTTGGAACCTCCT-3’  
              | R: 5’-TTGTCACACATCACCCAGGAG-3’ |

Statistical Analysis.

SPSS version 25.0 software was used for data analysis. The actual resistance rate of each antibiotic was selected for statistical analysis in this study.

Results and Discussion

Results

During the mentioned period of study, total of 86 Klebsiella species were isolated from collected clinical and non-clinical samples. K. pneumoniae represents the majority species isolated 39 (45.34%), followed by K. oxytoca 29 (33.72%) while K. planticola were 18 (20.9%).
prevalence of ESBL production in all Klebsiella species was 56 (65.11%) while K. pneumoniae ESBL production was 28 (71.7%) followed by K. oxytoca 5 (17.2%) and K. planticola was ESBL negative.

The samples Characteristics

Klebsiella spp. clinical samples were isolated from 29 male (54.71%) and 24 female (45.28%) patients. Most clinical samples of Klebsiella spp. were isolated from patients aged more than 20 years old (Table 2). Klebsiella spp. samples were mostly isolated from urine specimens (67.92%) and sputum (32.07%). Non-clinical isolates consider (60.6%) wastewater samples and 39.39% soil (Table 2).

Table 2 Characteristics of samples from which Klebsiella spp. were isolated

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Clinical Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>54.71</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>45.28</td>
</tr>
<tr>
<td>Clinical Specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>36</td>
<td>67.92</td>
</tr>
<tr>
<td>Sputum</td>
<td>17</td>
<td>32.07</td>
</tr>
<tr>
<td>• Non-clinical Specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wastewater</td>
<td>20</td>
<td>60.6</td>
</tr>
<tr>
<td>Soil</td>
<td>13</td>
<td>39.3</td>
</tr>
</tbody>
</table>

- The distribution of Klebsiella Species in different specimen sources.

Among the identified Klebsiella species, K. pneumoniae emerged as the dominant entity, constituting 41.5% of the aggregate Klebsiella spp. isolates from clinical specimen and 52.1% non-clinical sample (Table 3). In a closely comparable fashion, K. oxytoca displayed a substantial presence, encompassing 35.9% of the Klebsiella spp. isolates clinically and 31.6% others. A third discernible species, K. planticola, exhibited a significant but relatively less pronounced presence, accounting for 22.6% of the clinical isolates, 16.3% non-clinical one.

Table 3 Distribution of Klebsiella spp. in isolated samples

<table>
<thead>
<tr>
<th>Klebsiella Species</th>
<th>Specimens</th>
<th></th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>Clinical</td>
<td>22</td>
<td></td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>Non-clinical</td>
<td>17</td>
<td></td>
<td>51.51</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>Clinical</td>
<td>19</td>
<td></td>
<td>35.84</td>
</tr>
<tr>
<td></td>
<td>Non-clinical</td>
<td>10</td>
<td></td>
<td>30.3</td>
</tr>
<tr>
<td>K. planticola</td>
<td>Clinical</td>
<td>12</td>
<td></td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>Non-clinical</td>
<td>6</td>
<td></td>
<td>18.1</td>
</tr>
</tbody>
</table>
Figure 1: The phenotype of Klebsiella spp. colonies on several diagnostic media. A: Pink colonies on CHROMagar orientation medium. B: Bright metallic green colonies on Eosin Methylene Blue agar medium. C: Pink colonies on MacConkey agar medium. D: Milky white, non-hemolytic colonies on blood agar medium. E: Orange colonies on Hektoen Enteric agar medium.

Figure 2: Antibiotic Resistance Percentages for Klebsiella spp.
The phenotypic diagnosis of Metallo-\(\beta\)-Lactamases (MBLs) enzyme production in Klebsiella pneumoniae bacteria was achieved through the application of a specific methodology. The cornerstone of this approach was the utilization of the Double Disk Synergy Test (DDST) for diagnosis. The DDST method, recognized as one of the simplest techniques for phenotypically diagnosing MBLs enzymes, was implemented. This method involves the strategic placement of two disks containing Imipenem antigen (10 ng/disk). One of these disks was supplemented with EDTA. A positive outcome was identified by the discernible expansion of the inhibition zone surrounding the disk containing EDTA. Notably, this expansion was required to exceed 4 mm from the disk lacking EDTA. Figure 4 visual representation of this phenomenon.

The application of phenotypic diagnostic modalities facilitated the identification of a singular isolate within the cohort of K. pneumoniae bacteria. This isolate exhibited the capacity to produce Metallo-\(\beta\)-Lactamases enzymes. Importantly, this occurrence accounted for a proportion of 2.27% within the larger population, which encompassed a total of 22 isolates.

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**Figure 3: ESBLs enzyme resistance percentages for Klebsiella spp**

![Graph showing ESBL enzyme resistance percentages for Klebsiella spp](image-url)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>K. pneumoniae</th>
<th>K. oxytoca</th>
<th>K. planticola</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>PRL</td>
<td>PRL</td>
<td>AM</td>
</tr>
<tr>
<td>PRL</td>
<td>Piperacillin</td>
<td>K</td>
<td>Kanamycin</td>
</tr>
<tr>
<td>AMC</td>
<td>Amoxicillin-clavulanic acid</td>
<td>CTX</td>
<td>Cefotaxime</td>
</tr>
</tbody>
</table>
Figure 4: Using the DDST method used in phenotypic diagnosis to produce K. pneumoniae to MBL enzymes.

Genes encoding ESBL-CTX-M

All Klebsiella isolates investigated showed the presence of CTX-M gene. Additionally, the most frequent gene harbored isolates by K. pneumoniae (58%), of the K. oxytoca isolates (25%) recorded, followed by K. planticola (17%).

**Fig 5 RT-PCR for CTX-M gene for Klebsiella spp**

**Discussion**

In recent years, there has been an emergence of Klebsiella species strains integrating with devastating clinical outcomes.

The results of the present study revealed important insights into the distribution and prevalence of different Klebsiella species within the isolated cohort. Among the identified Klebsiella species, K. pneumoniae emerged as the dominant entity both in clinical and non-clinical specimens, constituting 41.5% and 51.5% respectively.

This finding aligns with previous studies that have highlighted the clinical significance and prevalence of K. pneumoniae as a major pathogen responsible for various infections, including pneumonia, sepsis, bloodstream infections, and urinary tract infections. The substantial
representation of K. pneumoniae isolates in this study emphasizes the need for effective strategies to control and manage infections caused by this bacterium [11].

Another notable finding was the substantial presence of K. oxytoca, which accounted for 35.9% of the clinical Klebsiella spp. isolates, furthermore 30.0% of environmental specimens. K. oxytoca is also recognized as an important pathogen associated with various infections. The high prevalence of K. oxytoca in this study, especially in clinical samples, suggests its potential role in the transmission of antibiotic resistance and highlights the need for further investigation into its virulence factors and resistance mechanisms.

In contrast, K. planticola exhibited a significant but relatively less pronounced presence, accounting for 22.6% and 18% of the isolates. While K. planticola is less dominant compared to K. pneumoniae and K. oxytoca, its presence is noteworthy and warrants further investigation. Previous studies have reported the isolation of K. planticola from human infections, indicating its potential as an opportunistic pathogen. Understanding the characteristics and virulence factors of K. planticola can contribute to a comprehensive understanding of the composition and distribution of Klebsiella species within the sampled population [12].

The study aimed to determine the prevalence of the antibiotic resistance profiling of Klebsiella spp., and the results provided valuable insights into the efficacy of various antibiotics in combating infections caused by this clinically significant bacterium. The high resistance percentages observed for antibiotics such as Ampicillin, Carbenicillin, and Kanamycin highlight the universal lack of efficacy of these antibiotics against Klebsiella pneumoniae. These findings align with the increasing concern of antibiotic resistance in Klebsiella pneumoniae, which has become a major public health threat. The varying levels of resistance observed for other antibiotics emphasize the complexity of treatment approaches necessary to effectively manage infections caused by this pathogen. The low resistance percentages observed for carbapenem antibiotics, such as Imipenem and Meropenem, indicate their potential efficacy against Klebsiella pneumoniae and highlight their importance as treatment options [13].

The present study also investigated the presence of Extended-Spectrum β-Lactamase (ESBLs) enzyme resistance and Metallo-β-Lactamases (MBLs) enzyme production in Klebsiella pneumoniae. The high ESBLs enzyme resistance percentages observed for various antibiotics further underscore the complexity of treatment options and the need for judicious antibiotic selection [11].

Sixty-five percent isolates of all Klebsiella spp. shows ESBL production which (71.7%) were Klebsiella pneumoniae. Therefore, the emergence and spread of ESBL-producing K. pneumoniae strains is a worrisome issue, as cephalosporins are ineffective against these isolates [14].

The absence of measurable MBLs enzyme resistance for Imipenem and Meropenem suggests their potential efficacy against Klebsiella pneumoniae, providing valuable insights for treatment strategies[15].

The findings of the present study align with previous studies that have highlighted the increasing concern of antibiotic resistance in Klebsiella pneumoniae [16]. The emergence of resistance to extended-spectrum cephalosporins has been a major concern, and the prevalence of ESBL-producing bacteria has been gradually increasing in acute care hospitals. The high ESBLs enzyme resistance percentages observed for various antibiotics in this study further emphasize the urgent need for effective strategies to combat antibiotic resistance in Klebsiella pneumoniae. The complexity of treatment options and the need for judicious antibiotic selection highlights the importance of developing novel approaches to combat infections caused by this pathogen.
The absence of measurable MBLs enzyme resistance for Imipenem and Meropenem suggests their potential efficacy against Klebsiella pneumoniae. This finding provides valuable insights for treatment strategies and emphasizes the importance of selecting appropriate antibiotics for the treatment of infections caused by this pathogen. The potential efficacy of Imipenem and Meropenem against Klebsiella pneumoniae aligns with previous studies that have highlighted the importance of carbapenem antibiotics as treatment options for infections caused by this pathogen.

The study also identified target CTX-M gene that is commonly associated with bacterial antibiotic resistance. Highly overexpressed CTX-M (Fig 5) can lead to high levels of resistance to extended-spectrum cephalosporin antibiotics, making it difficult to treat infections caused by these bacteria with conventional antibiotics (Fig 4).

High expression of CTX-M enzymes poses a serious challenge in healthcare settings because it can result in infections that are extremely difficult to treat with commonly used antibiotics. To combat this issue, healthcare professionals must use alternative antibiotics or combination therapies and take measures to prevent the spread of antibiotic-resistant bacteria, including proper hygiene practices and infection control protocols. Additionally, ongoing research and surveillance are crucial for monitoring the prevalence of high CTX-M expression and developing strategies to combat antibiotic resistance [17].

**Conclusion**

In conclusion, the results of present study provide a comprehensive understanding of the distribution and prevalence of different Klebsiella species within the isolated cohort. The findings highlight the dominance of K. pneumoniae and K. oxytoca, as well as the significant presence of K. planticola. The high resistance percentages observed for several antibiotics emphasize the urgent need for effective strategies to combat antibiotic resistance in Klebsiella spp. detected by both enzyme production and gene expression. These results contribute to a better understanding of the composition and distribution of Klebsiella species and provide valuable insights for clinical decision-making and treatment strategies. Further research is warranted to investigate the underlying molecular mechanisms driving resistance and to develop novel approaches to combat infections.

**References**

a tertiary care hospital. 2020.


